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# Direct Determination of Glyphosate, Glufosinate, and AMPA in Soybean and Corn by Liquid chromatography/tandem mass spectrometry

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## ABSTRACT

A simple high-throughput liquid chromatography/tandem mass spectrometry (LC-MS/MS) method was developed for the determination of glyphosate, glufosinate and aminomethylphosphonic acid (AMPA) in soybean and corn using a reversed-phase with weak anion-exchange and cation-exchange mixed-mode Acclaim™ Trinity™ Q1 column. Two grams of sample were shaken with ten milliliters of water containing ethylenediaminetetraacetic acid disodium salt ($Na_2EDTA$) and acetic acid for 10 min to precipitate protein. After centrifugation, the supernatant was passed thru an Oasis HLB SPE to retain suspended particulates and non-polar interferences. The sample was directly injected and analyzed in 6 min by LC-MS/MS with no sample concentration or derivatization steps. Two multiple reaction monitoring (MRM) channels were monitored in the method for each target compound to achieve true positive identification. Three internal standards corresponding to each analyte were used to counter matrix suppression effect. Linearity of the detector response with a minimum coefficient of determination ($R^2$) of more than 0.995 was demonstrated in the range of 10 to 1000 ng/mL for each analyte.

## INTRODUCTION

Glyphosate (N-phosphonomethyl glycine) and glufosinate [ammonium(S)-2-amino-4-[hydroxyl (methyl) phosphinoyl] butyrate] are non-selective post emergence herbicides used for the control of a broad spectrum of grasses and broad-leaf weed species in agricultural and industrial fields. AMPA is the major metabolite of glyphosate and also classified as a toxicologically significant compound (1). According to recent reports, there has been a dramatic increase in the usage of

these herbicides which are of risk to both human health and the environment (2). Glyphosate and glufosinate have high efficacy, low toxicity and an affordable price, when compared with other pesticides. These factors lead to its wide utilization on several crops. Farmers also use glyphosate as a desiccant to rapidly kill above ground growth of crops such as wheat. This allows for rapid dry down for easy harvest. Due to the low toxicity of glyphosate, the maximum residues levels (MRLs) established around the world are generally greater than the limits for other pesticides. According to FDA (40CFR180.364 and 40CFR180.364), the tolerance of glyphosate for soybean and corn are 20 and 5 µg/g and the tolerance of glufosinate in soybean and corn are 2 and 0.2 µg/g (3). However, some crops such as wheat and oats do not have a tolerance for glyphosate. Therefore, any glyphosate detected above the limit of quantification would be violative. A quick, accurate, and sensitive method to determine these herbicides in food grains must be developed to support the regulatory actions.

Glyphosate, glufosinate, and AMPA are very polar compounds and insoluble in organic solvents. These properties make the use of classical organic solvent extraction very difficult. Alferness and Iwata used an aqueous extraction method to extract glyphosate and AMPA from soil, plant and animal matrices (4). This method required the use of lengthy cleanup procedures that involved both anion and cation exchange columns. Typical silica based reversed-phase C18 columns experience difficulty with the retention of such polar compounds, and may generate non-resolved co-eluting peaks, often with polar analytes eluting in the void volume. The lack of chromophophore or fluorophore also necessitates the use of derivatization techniques for the determination of these analyte residues by liquid chromatography and gas chromatography (5-7). Vreeken and co-workers developed an analytical method to analyze glyphosate, AMPA and glufosinate in water samples using a reversed phase liquid chromatography separation after pre-column derivatization with 9-fluorenylmethyl chloroformate (FMOC-Cl) and detection by LC-MS/MS (8). Schreiber and Cabrices streamlined the derivatization by using a special autosampler for automation to determine these polar analytes in corn and soybean (9). The derivatization technique is not highly regarded by analysts as it requires the optimization of a number of parameters (temperature, reaction time, concentration and purity of the reagents, laboratory handling time). Anion exchange, Hydrophilic Interaction Liquid Chromatography (HILIC), and mixed-mode columns were used with LC-MS/MS to determined underivatized glyphosate and other polar pesticides in food matrixes with limited success (10,11,12).

This LIB describes a single laboratory validation of an LC-MS/MS method under a negative ion-spray ionization mode for the direct determination of glyphosate, glufosinate, and AMPA in soybean and corn. It also explains a quick and reliable extraction method that requires small sample size, non-toxic solvent, and an effective sample cleanup procedure to ensure a rugged, sensitive, and selective method.

## MATERIAL AND METHODS

### Chemicals and Materials

Pesticide standard (≥ 99% purity) were purchased from LGC Standards (Manchester, NH) consisting of glyphosate, AMPA, glufosinate, glyphosate $^{13}C2^{15}N$ (100 µg/mL), AMPA $^{13}C$ $^{15}N$ (100 µg/mL), and glufosinate D3. Methanol, acetonitrile, and water of HPLC grade were

obtained from Fisher Scientific (Pittsburgh, PA). Formic acid was obtained as 98% solution for mass spectrometry from Fluka (Buchs, Switzerland.). Acetic acid, Ammonium formate and Ethylenediaminetetraacetic acid disodium salt ($Na_2EDTA$) were purchased from Fisher Scientific (Pittsburgh, PA). Extracting solvent (50 mM acetic acid/10 mM $Na_2EDTA$) was prepared by mixing 572 µL of acetic acid and 0.74 g of $Na_2EDTA$ in 200-mL of purified water. Oasis HLB (60 mg) solid phase extraction cartridge was obtained from Waters (Milford, MA). EDP 3 electronic pipettes at different capacities (0-10 µL, 10-100 µL, and 100-1000 µL) were purchased from Rainin Instrument LLC (Oakland, CA) and were used for standard fortification.

A solution of 500 mM ammonium formate/formic acid (pH 2.9) was prepared as follows: 15.76 g of ammonium formate were dissolve in approximately 300 mL of HPLC water and adjusted with 98% formic acid (approx. 28.3 mL) until the pH reached 2.9 (using pH meter), and the solution was diluted to 500 mL with water. The HPLC mobile phase was prepared by mixing 100 mL of the 500 mM buffer solution with 900 mL of purified water so the final concentration was 50 mM.

**Standard Preparation**

The stock solution of glyphosate, glufosinate, and AMPA at 50, 10, and 1 µg/mL were prepared by dissolving the stock standard in 1:1 water:methanol solution. The solutions were maintained at 4 °C in polypropylene tubes to avoid adsorption to glass. The internal standard (IS) solution of glyphosate $^{13}C2^{15}N$, AMPA $^{13}C^{15}N$, and glufosinate D3 at 2 and 10 µg/mL were prepared by dissolving the stock standard in 1:1 water:methanol solution. The calibration standards were prepared in the extracting solvent or blank matrix extract (after SPE cleanup) with IS solutions for the calibration curves as described in Table 1.

**Sample Preparation and Extraction Procedure**

Organic soybean and corn were obtained from a local market. The samples were ground with a food processor until they had powder-like texture. The samples were weighed at 2 g each in 50-mL centrifuge tubes (Fisher Scientific, Pittsburgh, PA) and fortified with native standard solutions at 0.1, 0.5 and 2 µg/g (7 replicates) using Table 2. The IS solution (100 µL) at the concentration of 10 µg/mL was added into the samples so the concentration was 0.5 µg /g for all samples. The samples were allowed to stand at room temperature for 1 hour and then stored in a freezer overnight to let the analytes to be absorbed by the sample. A set of five non-fortified samples without IS were also prepared and used for matrix matched standard. On the extraction day, the spiked samples were allowed to thaw to room temperature. The extracting solvent (10 mL) was added to each tube using an automatic pipette. The tubes were capped tightly and shaken for 10 min on a SPEX 2000 Geno grinder (SPEX Sample Prep LLC, Metuchen, NJ) at 1000 stroke/min then centrifuged at 3,000 x g for 5 min using a Q-Sep 3000 centrifuge (Restek, Bellefonte, PA). Three milliliters of the supernatant were passed through an Oasis HLB cartridge (60 mg), previously conditioned with 2 mL methanol and 2 mL of the extracting solvent, and the last milliliter of the extract was collected into an autosampler vial. A 10 µL volume of sample was injected into the LC-MS/MS system.

**LC-MS/MS Analysis**

LC-MS/MS analysis was performed by using a Shimadzu HPLC system. The instrument was equipped with two LC-20AD pumps, a Sil-20AC autosampler, and a CTO-20AC column oven (Shimadzu, Kyoto, Japan), coupled with a 5500 Q-TRAP mass spectrometer from AB SCIEX (Foster City, CA). The Analyst software (version 1.6) was used for instrument control and data acquisition. Nitrogen and air from TriGas Generator (Parker Hannifin Co., Haverhill, MA) were used for nebulizer and collision gas in LC-MS/MS. An Acclaim™ Trinity™ Q1 (3 µm, 100 x 3 mm) from Thermo Scientific (Sunnyvale, CA) and a C18 SecurityGuard guard column (4 x 3 mm) from Phenomenex (Torrance, CA) were used for HPLC separation at 35 °C with sample injection volume of 10 µL. The mobile phase is 50 mM ammonium formate (pH 2.9) at a flow rate of 0.5 mL/min for a total run time of 6 min. The MS determination was performed in negative electrospray mode with monitoring of the two most abundant MS/MS (precursor/product) ion transitions using a scheduled MRM program of 60 seconds for each analyte. Analyte-specific MS/MS conditions and LC retention times for the analytes are shown in Table 3. The MS source conditions were as follows: curtain gas (CUR) of 30 psi, ion spray voltage (ISV) of -4500 volts, collisionally activated dissociation gas (CAD) is high, nebulizer gas (GS1) of 60 psi, heater gas (GS2) of 60 psi, source temperature (TEM) of 350 °C.

**RESULTS AND DISCUSSION**

**Chromatography Optimization**

Glyphosate, glufosinate, and AMPA possess negative charges in aqueous solution that make them difficult to be retained by a reversed-phase column. Several mixed phase mode columns containing reversed-phase, anion and cation exchange properties were evaluated for use in the study. They were a) Obelisc R (SIELC Technologies, Wheeling, IL ), zwitterionic-type mixed mode, b) Scherzo SM-C18 (Imtakt USA, Philadelphia, PA), mixed beads of cation and anion exchange particles, and c) Nanopolymer Silica Hybrid , Acclaim™ (Thermo Scientific, Sunnyvale, CA). Among the Acclaim™ columns, three different columns were also evaluated. They are Acclaim™ Trinity™ P1 (strong cation, weak anion/reversed-phase), Acclaim™ Trinity P2 (weak cation, strong anion/HILIC), and Acclaim™ Trinity™ Q1 (week cation, weak anion/reversed-phase). Since these columns have both cation and anion exchange properties, they are the ideal columns for the analysis of both cationic charge pesticides (paraquat, diquat, mepiquat, chlormequat, amitrole, and daminozide) and anionic charge pesticides (glyphosate, AMPA, glufosinate, forsetyl alumina, ethephon, and maleic hydrazide). The idea is to use a single column to determine all these very polar pesticides with one LC-MS/MS instrument.

After a lengthy column evaluation period, it was found the columns with strong cation exchange functionality would strongly retain paraquat and diquat. Therefore, they were not considered as the column of choice. Different mobile phase parameters were evaluated which included pH (2.8 to 5), acetonitrile concentration (0 – 100%), and salt concentration (0 – 100 mM). The best column so far was the Acclaim™ Trinity™ Q1 which provided good peak shape and reasonable retention for all analytes. The most important parameter was the pH of the mobile phase. At low

pH (2.9), glyphosate eluted well while paraquat and diquat were strongly retained. At higher pH (3.5), glyphosate was a late eluter with a wide and tailing peak shape while paraquat and diquat had good peak shape. Therefore, two analyses on a single column should be done isocratically with two different mobile phases. Higher acetonitrile content in the mobile phase enhanced sensitivity and increased the retention time of the analytes. If too high, the acetonitrile content in the mobile phase resulted in very broad and late-eluting glyphosate peak at pH 2.9. High salt concentration shortened the retention time of the analytes and decreased analyte response due to ion-suppression. All of these three parameters must be chosen appropriately to achieve optimal separation and peak sensitivity for the target analytes.

It was found that the mobile phase containing 50 mM ammonium formate (pH 2.9) at a flow rate of 0.5 mL/min for the Acclaim™ Trinity™ Q1 (3 μm, 100 x 3 mm) produced the optimal conditions for peak shape, retention time, and sensitivity for these three analytes.

**Optimization of Sample Extraction Procedure**

For high protein sample such as soybean, protein precipitation is a common protocol for rapid sample clean-up and extraction (13). An organic solvent and acid have been used for effecting protein precipitation by exerting specific interactive effects on the protein structure. An organic solvent lowers the dielectric constant of the protein solution and also displaces the ordered water molecules around the hydrophobic regions on the protein surface, the former enhancing electrostatic attractions among charged protein molecules and the latter minimizing hydrophobic interactions among the proteins. Acidic reagents form insoluble salts with the positively charged amino groups of the proteins at pH values below their isoelectric points. EDTA was used to improve extracting efficiency of tetracycline in milk (14,15,16). Aqueous solution containing 50 mM acetic acid/10 mM $Na_2EDTA$ was successfully used in extracting glyphosate, glufosinate, and AMPA in milk sample with recovery over 90% (17). Acetic acid lowered the pH of the sample to precipitate the protein and $Na_2EDTA$ prevented chelation complex between polyvalent metal ions in the sample and the analytes.

Lecithin is a phospholipid found in soybeans that could be extracted along with the analytes in aqueous solution. They may accumulate at the head of the analytical column under high aqueous mobile phase condition and degrade column performance. Therefore, the Oasis HLB cartridge was added to the method to filter the aliquot and trap the phospholipid and other non-polar compounds in the final extract. Special cleanup cartridges specifically designed for phospholipids such as Captiva (Agilent Technology, Santa Clara, CA ) and HybridSPE-plus (Supelco, Bellefonte, PA) were also evaluated with poor recovery because glyphosate and glufosinate have phosphate functional groups similar to those in phospholipids.

To evaluate the optimal extraction time, a soybean sample containing incurred residue of glyphosate (~10 μg/g) was put in five 50-mL plastic centrifuge tubes and 10 mL of the extracting solvent was added into each tube. The tubes were shaken on the SPEX 2000 Geno grinder at 1000 stroke/min at 2, 5, 10, 30, and 60 min, and then centrifuged at 3000 x g for 5 min using the Q-Sep 3000 centrifuge. The supernatant was passed thru an Oasis HLB cartridge (60 mg), previously conditioned with 2 mL methanol and 2 mL of the extracting solvent, and the last milliliter of the extract was collected into an autosampler vial. Ten microliters of the sample

extract were injected into the LC-MS/MS system. The results showed that there was no significant difference in glyphosate concentration in sample extract after the samples were shaken at 5, 10, 30, and 60 min. At 2 min of shaking, the concentration of glyphosate was approximately 70% of the sample shaken at 5 min. This suggested that five minute was long enough to extract glyphosate effectively. However, the ten minutes extraction time was chosen as the optimum extraction time for this method.

**Evaluation of Matrix Effects**

Matrix effect (%ME) in the sample extract was calculated as the slope of calibration curve of analyte in sample matrix divided by the slope of calibration curve of analyte in solvent and multiplied by 100 (Figure 1) . Therefore, a value of 100% means that no matrix effect is present. If the value is less than 100%, it means that there is matrix suppression. If the value is more than 100%, it means that there is matrix enhancement. Table 4 shows the %ME of all three analytes in both matrices. Glyphosate had minimum degree of suppression (95-101 %) in both matrices, while AMPA had severe suppression (17- 30%). Glufosinate has less % ME in soybean (74%) than in corn (92%). Based on this data, IS may not be needed for glyphosate and glufosinate analysis in soybean and corn (reduces the cost of analysis). However, it is necessary to use IS for AMPA analysis to correct for matrix suppression.

**Method Validation**

The calibration standard solutions at concentrations from 10 to 1000 ng/mL were prepared in both sample matrices (soybean and corn) and extracting solvent with the addition of IS (Table 1). These standard solutions were injected along with the fortified samples and sample blank as described in the Table 2. For comparison purposes, four different quantification methods were used to determine the accuracy and precision of the recovery results. They were a) standard in matrix with internal standard calibration method, b) standard in matrix with external calibration method, c) standard in solvent with internal standard calibration method, and d) standard in solvent with external standard calibration method (Table 5). The linearity was evaluated and they showed satisfactory linearity with coefficient of determination ($R^2$) of more the 0.995. The specificity of the method was evaluated by analyzing reagent blank, blank sample and blank sample spiked at the lowest fortification level (0.1µg/g). No relevant signal (above 30%) was observed at any of the transitions selected in the blank sample. A reagent blank was injected immediately after the 1000 ng/mL standard and no analyte signals were detected above 10% of the 10 ng/mL standard.

The method detection limit (MDL) for each compound was calculated according to FDA guidelines with 7 replicates of the lowest calibration standard (10 ng/mL). The MDL was calculated by multiplying standard deviation of 7 replicates with t value at a degree of freedom of 6 (3.14). By using matrix matched standard with IS, the MDL for glyphosate, glufosinate, and AMPA were 2.3, 2.3, and 4 ng/mL for soybean sample and 2.0, 4.8, and 5.5 ng/mL for corn sample, respectively. The method quantification limit (MQL) was three times the MDL which were 6.9, 6.9, and 11.9 ng/mL for soybean and 5.9, 14.4, and 16.5 ng/mL for corn, respectively.

Accuracy (recovery %) and precision (relative standard deviation or RSD %) were evaluated at the fortification levels of 0.1, 0.5, and 2 ng/g in seven replicates in both soybean and corn samples (Table 7 and 8) using all 4 calibration methods. For glyphosate and glufosinate, the average recovery using a) standard in matrix with internal standard calibration method, b) standard in matrix with external calibration method, and c) standard in solvent with internal standard calibration method was in the range of 92-104% with the RSD of less than 6 %. The calibration of standard in solvent without the IS had average recovery ranged from 96-98% with the RSD of less than 5% for glyphosate. However, it had average recovery range from 75-76 % with the RSD of less than 5%. This demonstrates that glyphosate can be effectively extracted from the sample and does not have significant matrix suppression. External standard calibration without the IS can used to accurately quantify glyphosate in these samples. On the other hand, IS should be used to accurately quantify glufosinate to compensate for the matrix suppression

The recovery of AMPA using calibration curve without IS in both matrices were very low due to matrix suppression as expected from the results in Table 4. The calibration curve from matrix match standard (without IS) improves the recovery of AMPA somewhat, but it is still less than 70%. AMPA was eluted near the solvent front where polar interferences in the matrix were present. The concentration of these interferences was not predictable depending upon the type of matrix. Therefore, the IS (AMPA $^{13}C$ $^{15}N$) should be used to accurately quantify AMPA in these samples. The recovery of AMPA using IS in sample matrix and in solvent were in the range of 96-113% with the RSD of less than 12% in both matrices. Therefore, standard in solvent with IS may be used for the quantification of AMPA to save time and cost of analysis.

Chromatograms of glyphosate, glufosinate, and AMPA in soybean blank and soybean blank fortified at 0.1 µg/g are shown in Figures 3 and 4. Chromatograms of glyphosate, glufosinate, and AMPA in corn blank and corn blank fortified at 0.1 µg/g are shown in Figures 5 and 6. No significant inferences were observed the blank sample where the analytes were eluted. The Acclaim™ Trinity Q1 combined reverse-phase, weak anion, and weak cation exchange properties in one column. This column retained glyphosate, glufosinate, and AMPA by the ion-exchange mechanism similar to the previous work done by Hao et. al. on the Acclaim™ WAX-1 column (9). However, a lower concentration of salt in the mobile phase (50 mM ammonium formate) at a much lower pH, significantly improved peak shape and sensitivity with simple isocratic elution. The column was rugged and gave good peak shape and retention time reproducibility over 100 injections of sample matrix without the need for column reconditioning as previously recommended by Hao and coworkers.

A soybean sample and a corn sample collected from the market that contained incurred residue were analyzed by this method. The soybean sample contained 11 ppm of glyphosate and 4.9 ppm of AMPA (Figure 7). The corn sample contained 6.5 ppm of glyphosate and 0.065 ppm of AMPA (Figure 8). There was no glufosinate detected above 0.03 ppm in either sample.

## CONCLUSION

This work describes a ten-minute extraction with aqueous solution of acetic acid and $Na_2EDTA$ which allows a rapid and direct determination of glyphosate, glufosinate, and AMPA residue in soybean and corn samples. Acetic acid precipitates soluble protein (major interference) from the

sample extract while $Na_2EDTA$ prevents the analytes from forming a chelation complex with polyvalent metal. Oasis HLB SPE is used to filter the sample extract and trap the phospholipids and other non-polar compounds. The SPE cleanup step is used to maintain HPLC column performance and minimize matrix concentration in the final extract. The mixed-mode Acclaim™ Trinity™ Q1 HPLC column allows the analytes to be retained on the column and separated from each other without a derivatization step. These analytes were commonly derivatized before HPLC analysis to improve their chromatographic retention in reversed-phase LC. Negative mode ion-spray with MS/MS measurement gives excellent sensitivity and selectivity that produce distinct chromatographic peaks with minimal interference. Severe matrix effect on AMPA was clearly observed because it co-eluted with other polar interferences near the solvent front. The use of isotope-labeled AMPA eliminates the matrix suppression problem and provides accurate quantification.

The proposed method is quick, rugged, selective, and sensitive enough to determine glyphosate, glufosinate and AMPA in soybean, corn and other food grains at or above the 50 ng/g level . It can be used as an alternate method to the traditional FMOC-bases methods which require tedious and time-consuming derivatization and concentration steps.

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Table 1. Preparation of Calibration Standard.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| sample extract or extracting solvent (µL) | 425 | 425 | 425 | 425 | 425 | 425 | 425 |
| extracting solvent (µL) | 45 | 37.5 | 25 | 0 | 37.5 | 25 | 0 |
| pesticide mix 1 µg/mL (µL) | 5 | 12.5 | 25 | 50 | 0 | 0 | 0 |
| pesticide mix 10 µg/mL (µL) | 0 | 0 | 0 | 0 | 12.5 | 25 | 50 |
| IS 2 µg/mL (µL) | 25 | 25 | 25 | 25 | 25 | 25 | 25 |
| total volume (µL) | 500 | 500 | 500 | 500 | 500 | 500 | 500 |
| IS concentration (ng/mL) | **100** | **100** | **100** | **100** | **100** | **100** | **100** |
| final concentration (ng/mL) | **10** | **25** | **50** | **100** | **250** | **500** | **1000** |
| | | | | | | | |

Table 2. Preparation of fortified samples (for each 2 g of sample and a final volume of 10 mL)

| fortification level | standard mix | standard mix | IS mix 10 µg/mL | expected conc. |
|---|---|---|---|---|
| (µg/g) | 10 ng/ µL (µL) | 50 ng/ µL (µL) | (µL) | in the extract (ng/mL) |
| | | | | |
| 0 | 0 | 0 | **100** | **0** |
| 0.1 | 20 | 0 | **100** | **20** |
| 0.5 | 100 | 0 | **100** | **100** |
| 2.0 | | 80 | **100** | **400** |

Table 3. Retention time and MRM conditions for LC-MS/MS analysis.

| Analyte | Precursor Ion (m/z) | Product Ion (m/z) | DP | CE | EP | CXP | Retention Time (min) |
|---|---|---|---|---|---|---|---|
| AMPA.1 | 110 | 63 | -60 | -24 | -10 | -10 | 1.1 |
| AMPA.2 | 110 | 79 | -60 | -26 | -10 | -10 | 1.1 |
| AMPA $^{13}C^{15}N$ (IS) | 112 | 63 | -60 | -24 | -10 | -10 | 1.1 |
| Glufosinate.1 | 180 | 95 | -46 | -23 | -10 | -10 | 1.65 |
| Glufosinate.2 | 180 | 85 | -46 | -26 | -10 | -10 | 1.65 |
| Glufosinate D3 (IS) | 183 | 63 | -46 | -26 | -10 | -10 | 1.65 |
| Glyphosate.1 | 168.2 | 63 | -110 | -30 | -10 | -10 | 2.05 |
| Glyphosate.2 | 168.2 | 79 | -110 | -55 | -10 | -10 | 2.05 |
| Glyphosate $^{13}C2^{15}N$ (IS) | 171 | 63 | -110 | -30 | -10 | -10 | 2.05 |

Compound dependent parameters: DP = declustering potential, CE = collision energy, EP = entrance potential, CXP = collision cell exit potential

Table 4. Matrix effect evaluation soybean (using calibration curve with linear fit)

**Soybean**

| | Slope of cal. curve in solvent | Slope of cal. curve matrix | Matrix effect (%ME) |
|---|---|---|---|
| glyphosate | 772 | 731 | 95 |
| glufosinate | 755 | 562 | 74 |
| AMPA | 1499 | 258 | 17 |

**Corn**

| | Slope of cal. curve in solvent | Slope of cal. curve matrix | Matrix effect (%ME) |
|---|---|---|---|
| glyphosate | 812 | 823 | 101 |
| glufosinate | 779 | 718 | 92 |
| AMPA | 1516 | 455 | 30 |

Table 5. Linear regression of the calibration curve (1/x weighing) using four different methods (soybean).

| Analyte | Calibration curve type | Slope | intercept | coefficient of determination (R2 ) |
|---|---|---|---|---|
| **glyphosate** | Matrix with IS | 0.0156 | 0.0392 | 0.9995 |
| | Matrix without IS | 733 | 1720 | 0.9999 |
| | Solvent with IS | 0.0158 | 0.0371 | 0.9985 |
| | Solvent without IS | 765 | 1770 | 0.9998 |
| **glufosinate** | Matrix with IS | 0.0151 | 0.0189 | 0.9994 |
| | Matrix without IS | 559 | 1240 | 0.9998 |
| | Solvent with IS | 0.0158 | 0.00773 | 0.9994 |
| | Solvent without IS | 760 | 365 | 0.9996 |
| **AMPA** | Matrix with IS | 0.0436 | 2.72 | 0.9987 |
| | Matrix without IS | 261 | 12400 | 0.9991 |
| | Solvent with IS | 0.0413 | 2.72 | 0.9985 |
| | Solvent without IS | 1480 | 81000 | 0.9991 |

Table 6. Linear regression of the calibration curves (1/x weighing) using four different methods (corn).

| Analyte | Calibration curve type | Slope | Intercept | Coefficient of determination ($R^2$) |
|---|---|---|---|---|
| **Glyphosate** | Matrix with IS | 0.0156 | 0.0597 | 0.9985 |
| | Matrix without IS | 796 | 3750 | 0.9996 |
| | Solvent with IS | 0.0158 | 0.0378 | 0.9993 |
| | Solvent without IS | 791 | 1310 | 0.9993 |
| **Glufosinate** | Matrix with IS | 0.0153 | 0.0722 | 0.9979 |
| | Matrix without IS | 711 | 1800 | 0.987 |
| | Solvent with IS | 0.0157 | 0.0126 | 0.9999 |
| | Solvent without IS | 763 | 546 | 0.9994 |
| **AMPA** | Matrix with IS | 0.0439 | 2.92 | 0.9986 |
| | Matrix without IS | 474 | 48200 | 0.9989 |
| | Solvent with IS | 0.0423 | 2.77 | 0.9993 |
| | Solvent without IS | 1500 | 84500 | 0.9993 |

Table 7. Recovery (%) and RSD (%) data obtained in the validation experiments (soybean) (n = 7)

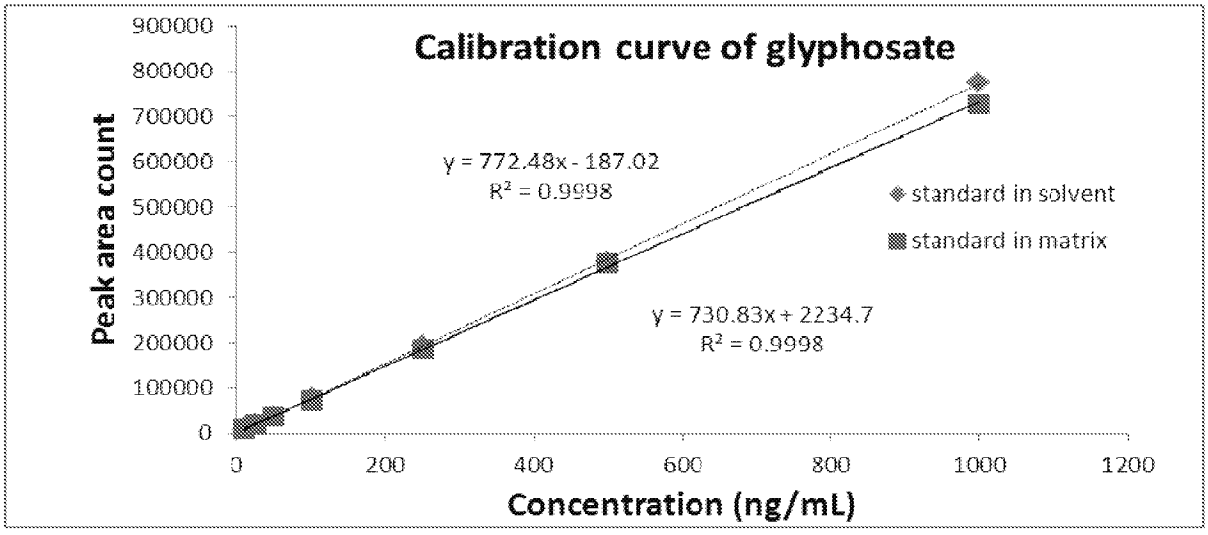
| Analyte | Fortification level (µg/g) | | Calibration method | | | |
|---|---|---|---|---|---|---|
| | | | **Matrix with IS** | **Matrix no IS** | **Solvent with IS** | **Solvent no IS** |
| **Glyphosate** | 0.1 | Recovery (%) | 103 | 101 | 102 | 97 |
| | | RSD (%) | 4.26 | 4.72 | 3.34 | 4.66 |
| | 0.5 | Recovery (%) | 102 | 100 | 101 | 96 |
| | | RSD (%) | 3.98 | 2.96 | 3.51 | 3 |
| | 2 | Recovery (%) | 102 | 103 | 100 | 98 |
| | | RSD (%) | 2.43 | 3.07 | 2.36 | 3 |
| **Glufosinate** | 0.1 | Recovery (%) | 102 | 95 | 101 | 76 |
| | | RSD (%) | 4.28 | 5.2 | 4.13 | 4.86 |
| | 0.5 | Recovery (%) | 102 | 100 | 98 | 75 |
| | | RSD (%) | 3.95 | 1.6 | 3.83 | 1.69 |
| | 2 | Recovery (%) | 98 | 104 | 94 | 76 |
| | | RSD (%) | 2.99 | 3.85 | 3.07 | 3.75 |
| **AMPA** | 0.1 | Recovery (%) | 101 | 57 | 106 | NA |
| | | RSD (%) | 6.3 | 28.83 | 4.53 | NA |
| | 0.5 | Recovery (%) | 108 | 78 | 107 | NA |
| | | RSD (%) | 6.35 | 5.76 | 4.36 | NA |
| | 2 | Recovery (%) | 105 | 80 | 108 | 2 |
| | | RSD (%) | 7.59 | 11.21 | 5.85 | 63.53 |

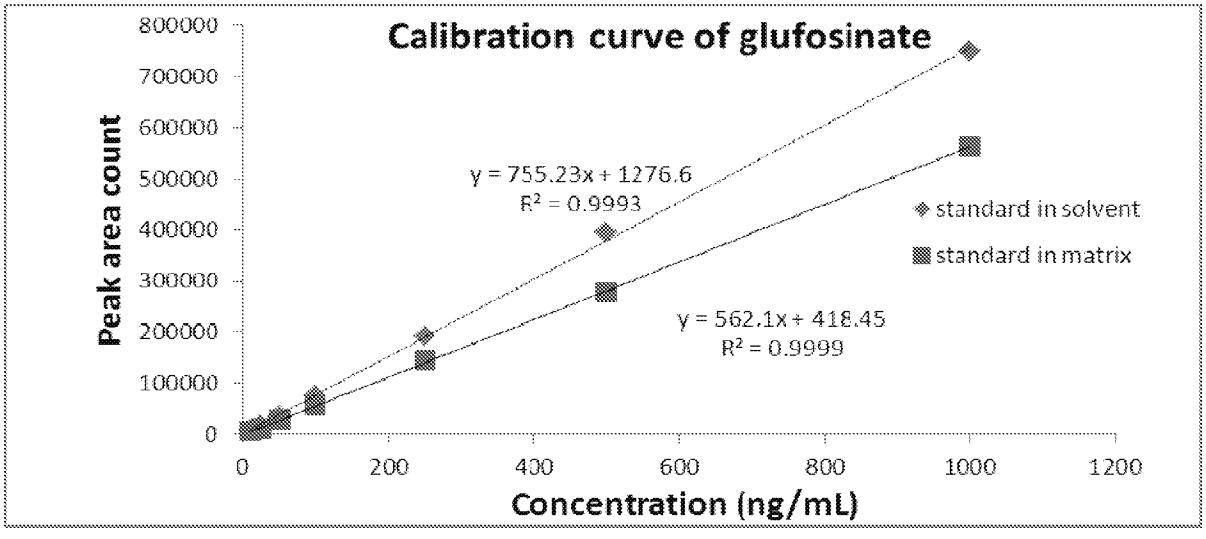
Table 8. Recovery (%) and RSD (%) data obtained in the validation experiments (n = 7).

(Corn)

| Analyte | Fortification level (µg/g) | | Calibration method | | | |
|---|---|---|---|---|---|---|
| | | | **Matrix with IS** | **Matrix no IS** | **Solvent with IS** | **Solvent no IS** |
| **Glyphosate** | 0.1 | Recovery (%) | 100 | 89 | 104 | 105 |
| | | RSD (%) | 4.78 | 6.3 | 3.59 | 5.4 |
| | 0.5 | Recovery (%) | 104 | 96 | 104 | 99.4 |
| | | RSD (%) | 4.24 | 4.0 | 4.18 | 3.9 |
| | 2 | Recovery (%) | 107 | 97 | 106 | 98 |
| | | RSD (%) | 3.79 | 2.7 | 3.77 | 2.8 |
| **Glufosinate** | 0.1 | Recovery (%) | 92 | 96 | 99 | 97 |
| | | RSD (%) | 8.64 | 9.9 | 4.8 | 9.1 |
| | 0.5 | Recovery (%) | 103 | 99 | 104 | 94 |
| | | RSD (%) | 3.98 | 3.7 | 3.7 | 3.6 |
| | 2 | Recovery (%) | 103 | 99 | 101 | 92 |
| | | RSD (%) | 5.29 | 3.4 | 5.25 | 3.3 |
| **AMPA** | 0.1 | Recovery (%) | 96 | NA | 113 | NA |
| | | RSD (%) | 11.96 | NA | 6.48 | NA |
| | 0.5 | Recovery (%) | 103 | 8.2 | 111 | NA |
| | | RSD (%) | 8.26 | 48.6 | 7.8 | NA |
| | 2 | Recovery (%) | 105 | 52 | 110 | 10.4 |
| | | RSD (%) | 6.89 | 5.8 | 6.95 | 9.28 |

Figure 1. Calibration curves of analytes in solvent and in blank soybean matrix



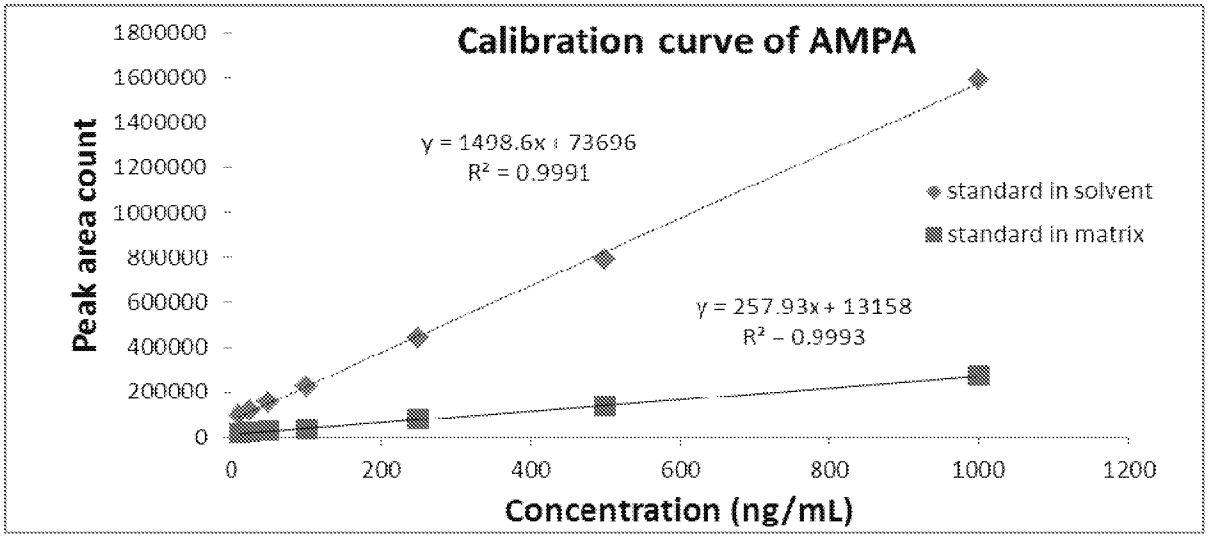
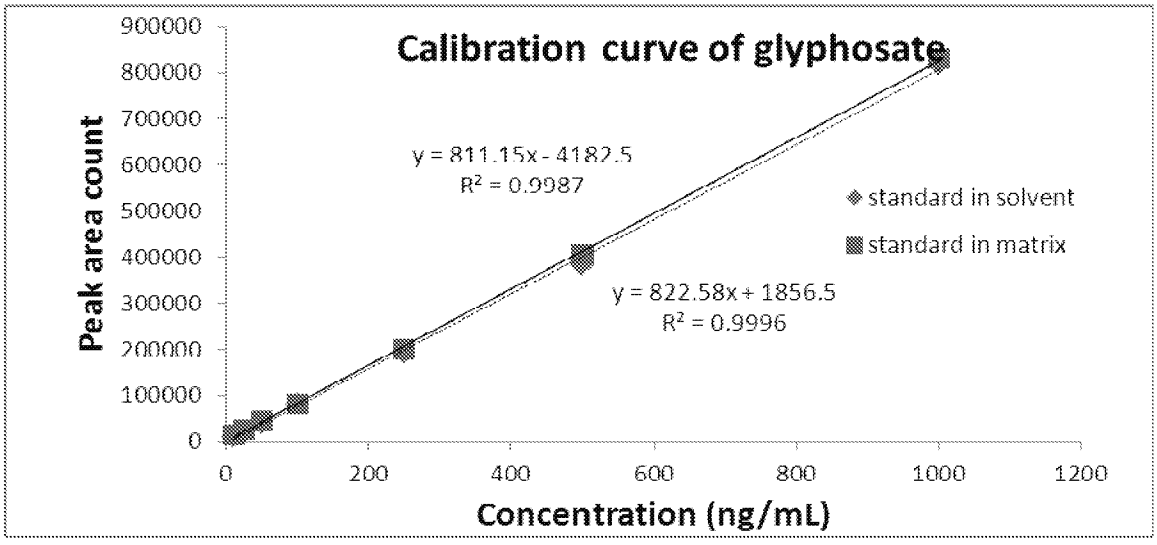
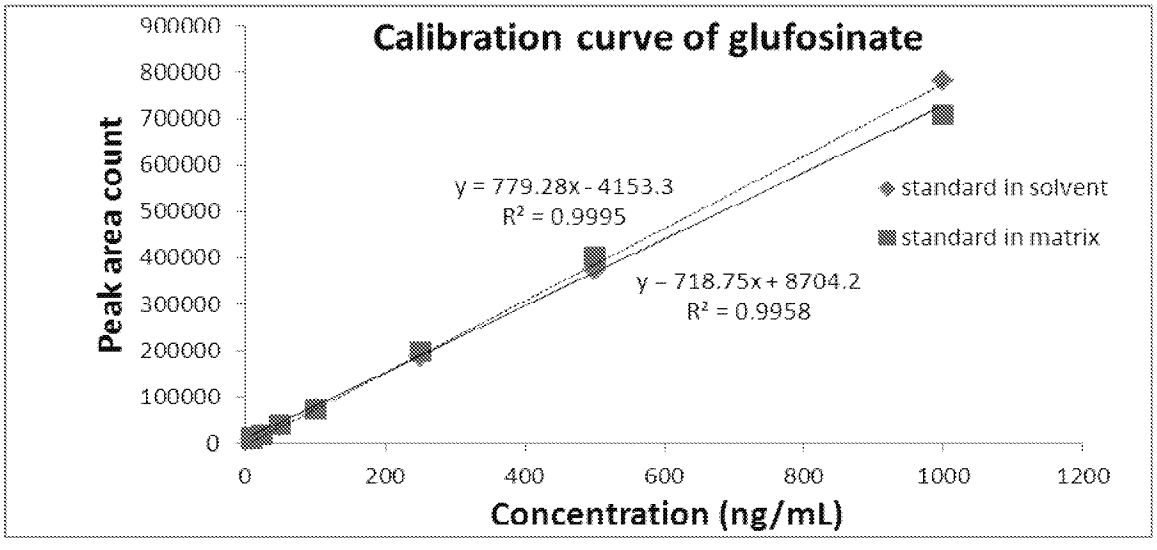



Figure 2. Calibration curves of analytes in solvent and in blank corn matrix



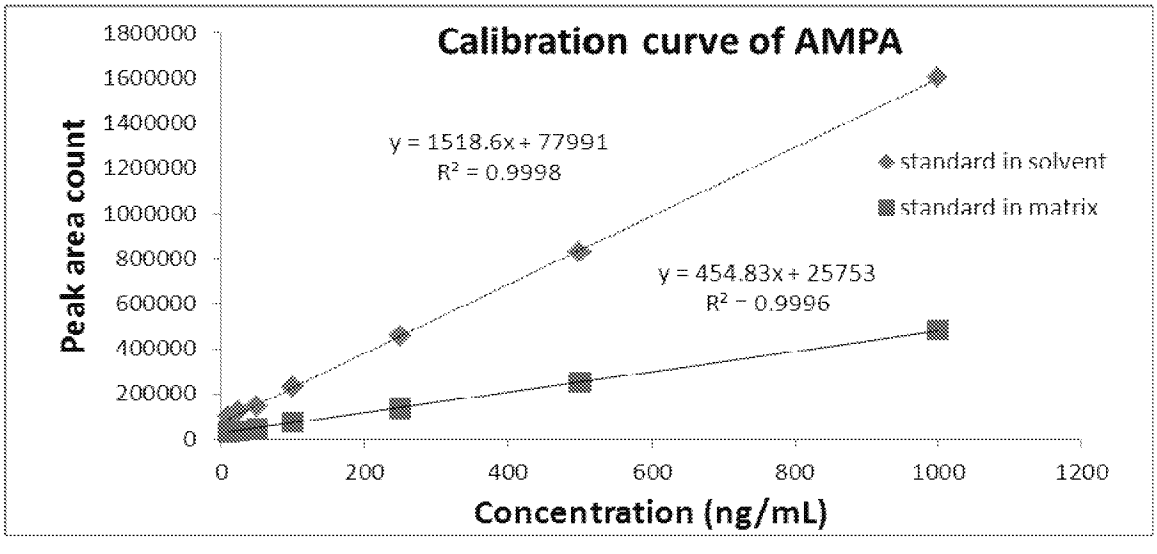
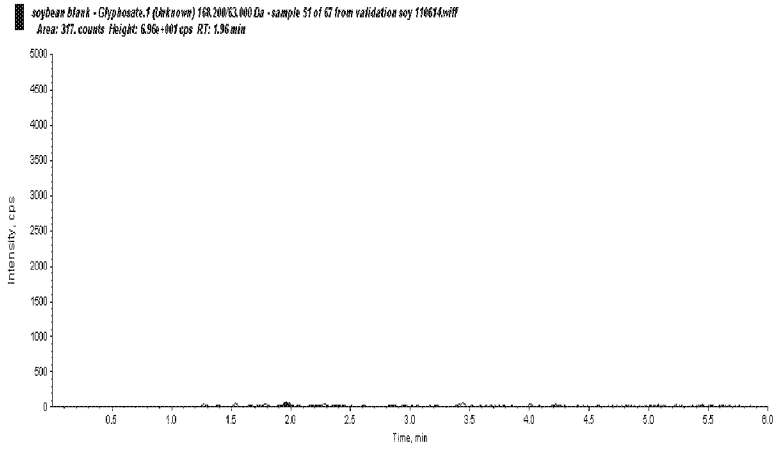
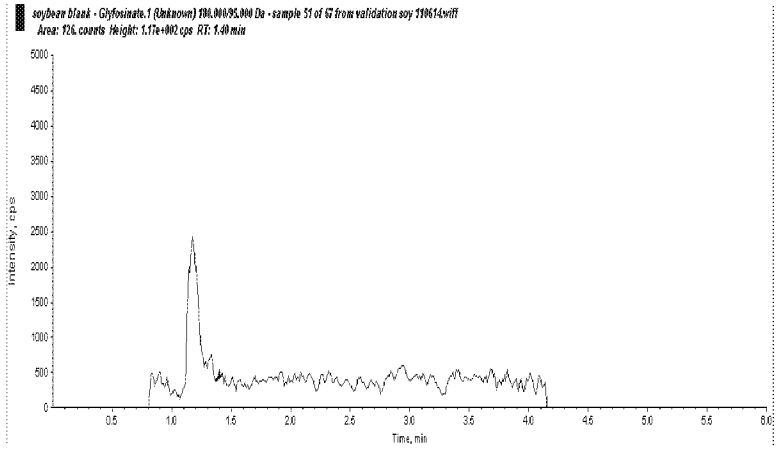



Figure 3 Chromatogram of soybean blank

Glyphosate channel




Glufosinate channel




AMPA channel

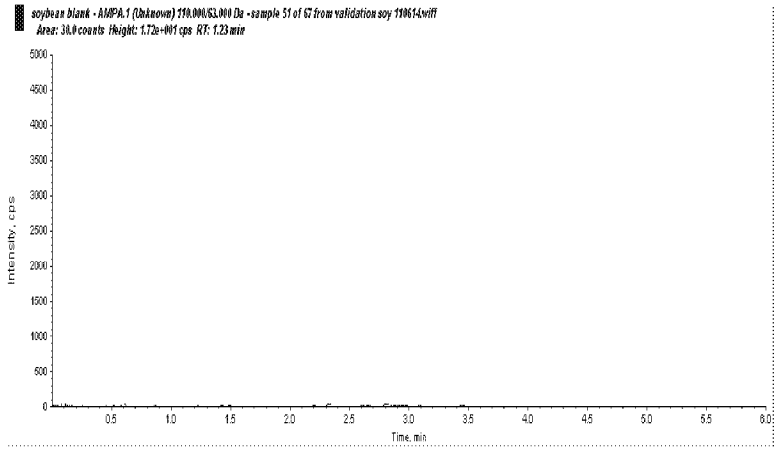
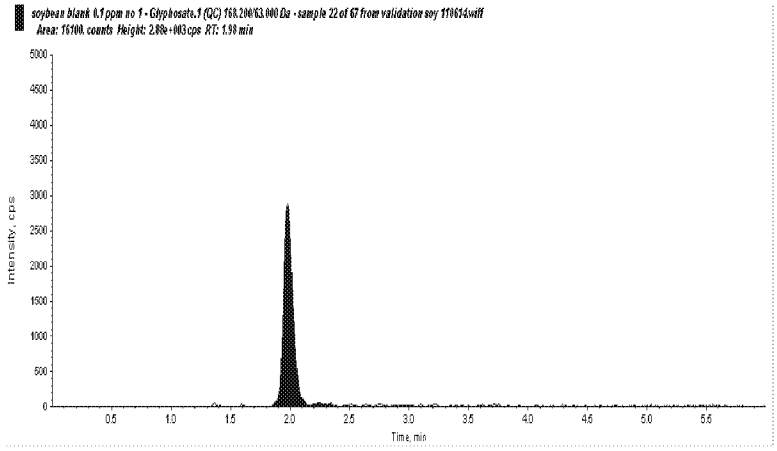
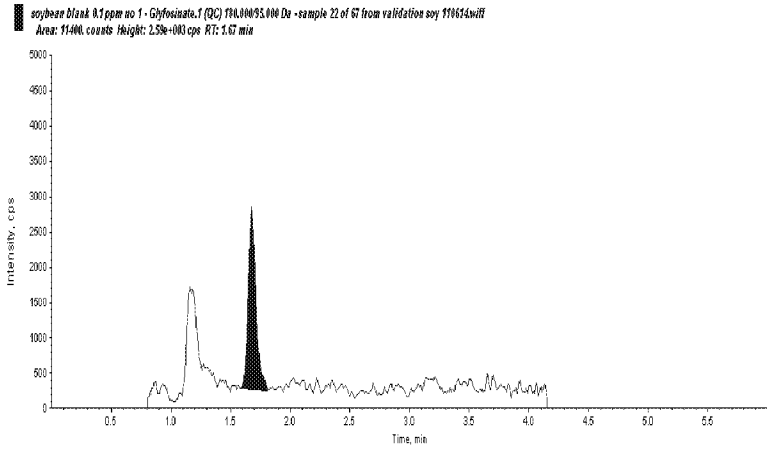


Figure 4 Chromatogram of soybean blank fortified at 0.1 ng/g of glyphosate, glufosinate and AMPA

Glyphosate channel




Glufosinate channel




AMPA channel

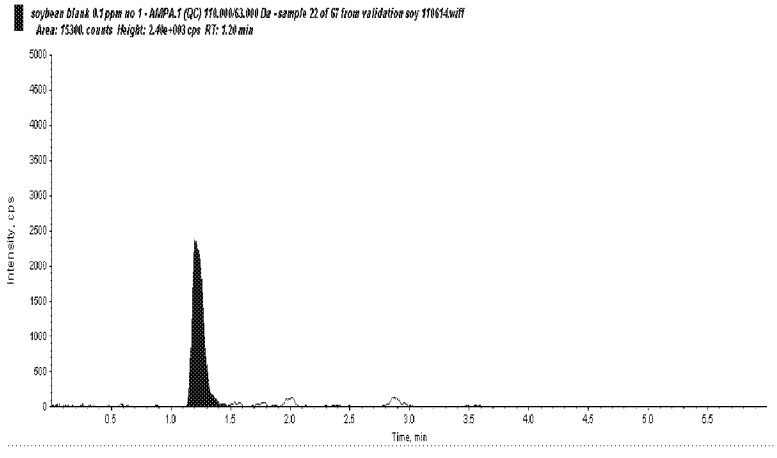
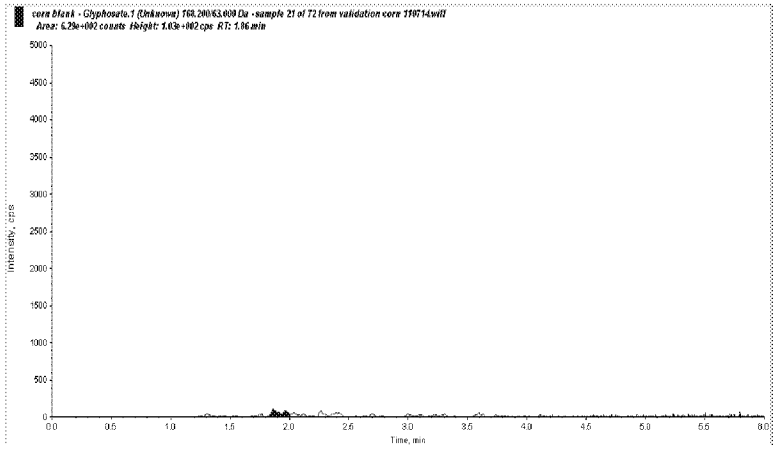
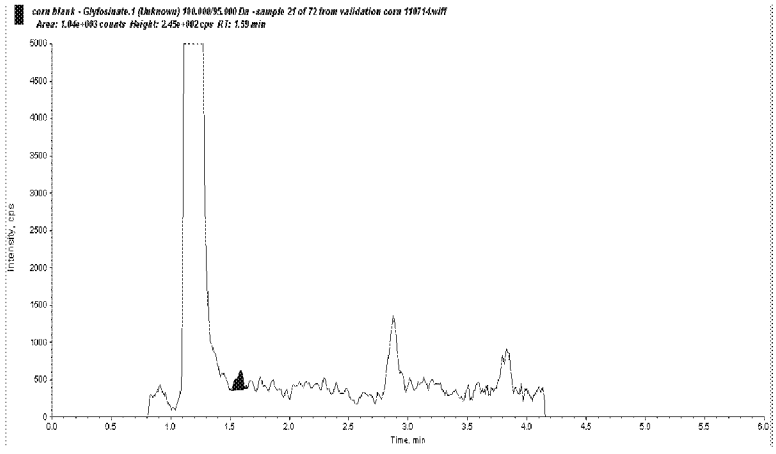


Figure 5 Chromatogram of corn blank

Glyphosate channel




Glufosinate channel




AMPA channel

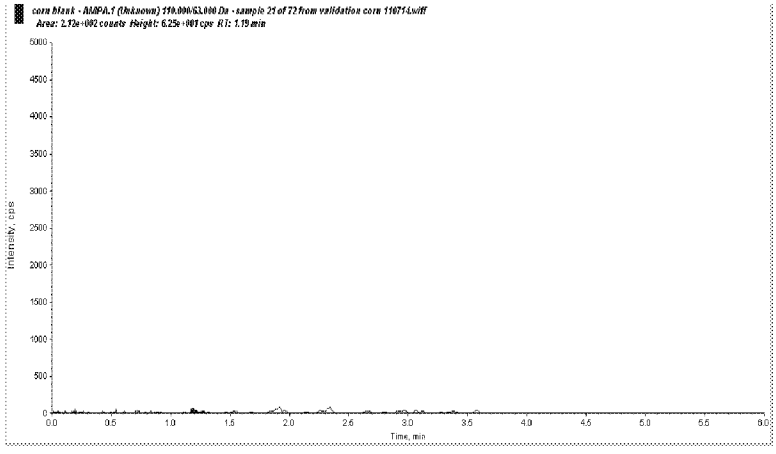
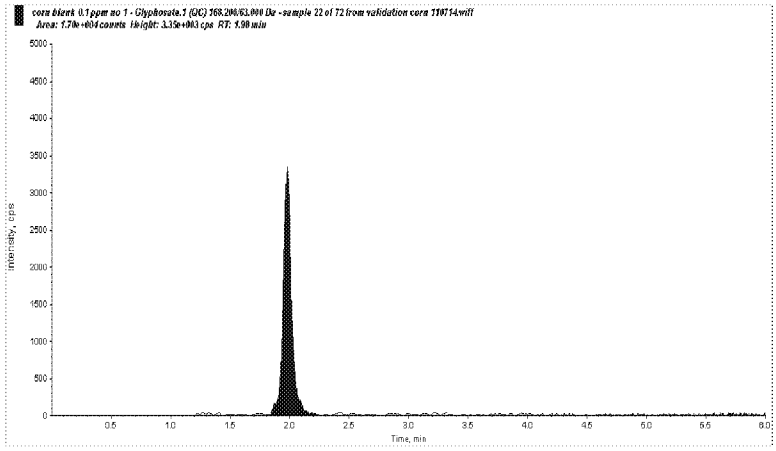
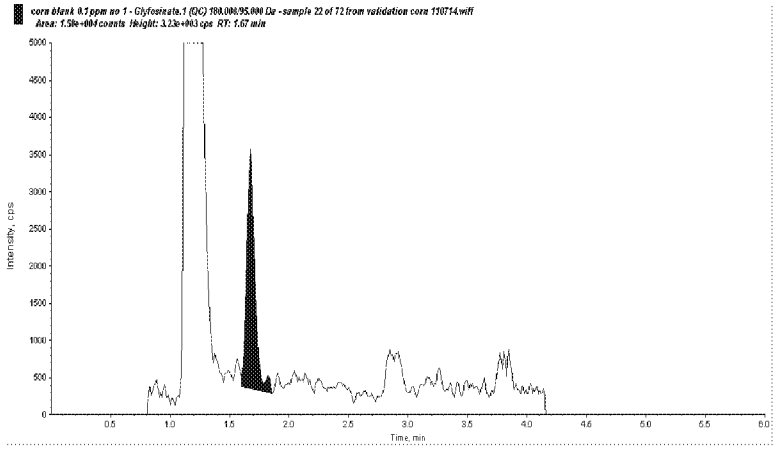


Figure 6 Chromatogram of corn blank fortified at 0.1 ng/g of glyphosate, glufosinate and AMPA

Glyphosate channel




Glufosinate channel




AMPA channel

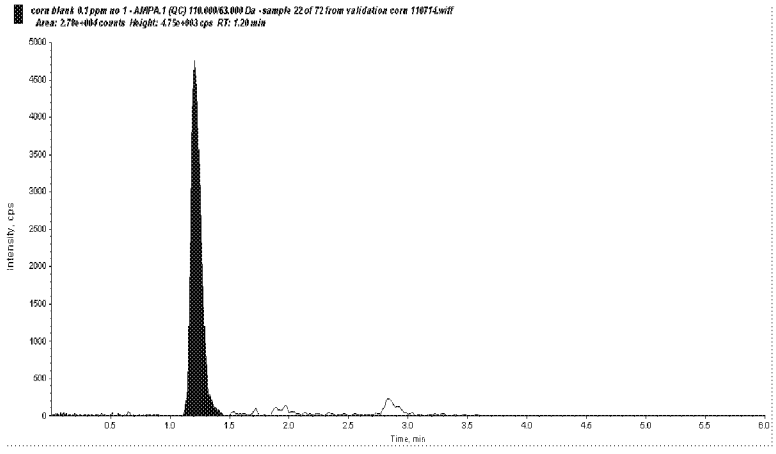
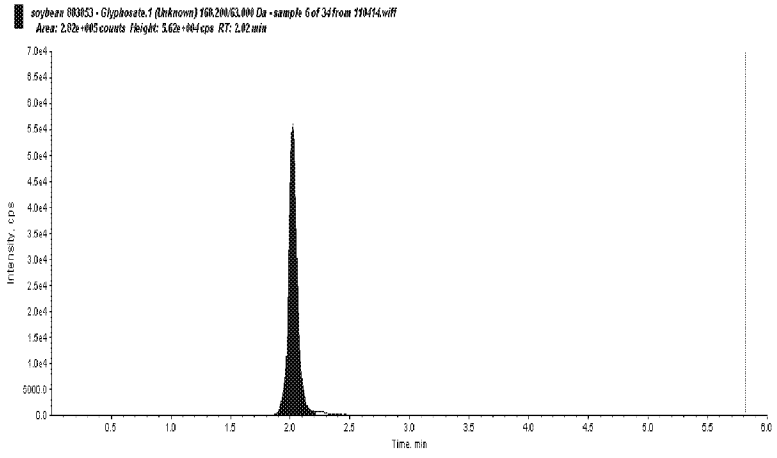
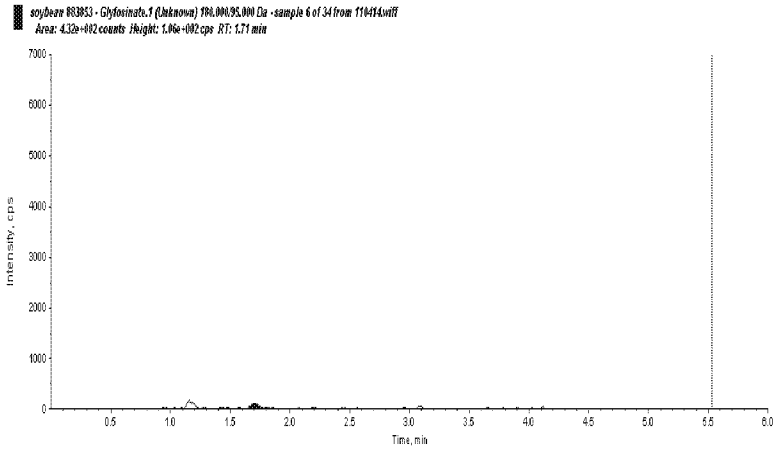


Figure 7 Chromatogram of soybean containing 11.0 ppm of glyphosate and 4.9 ppm of AMPA

Glyphosate channel




Glufosinate channel




AMPA channel

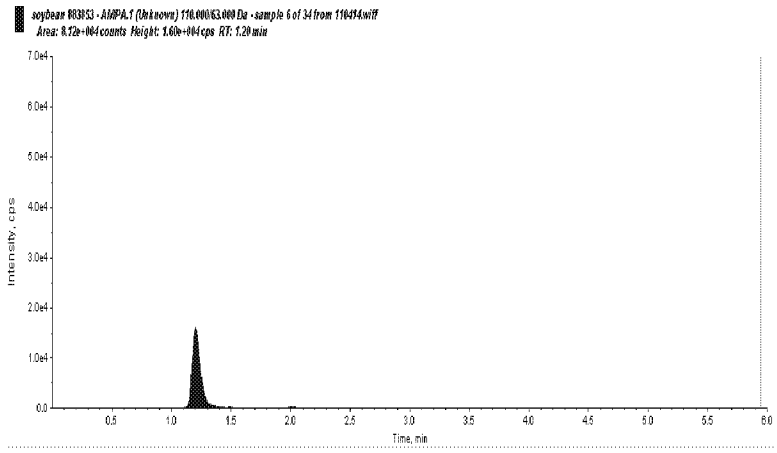
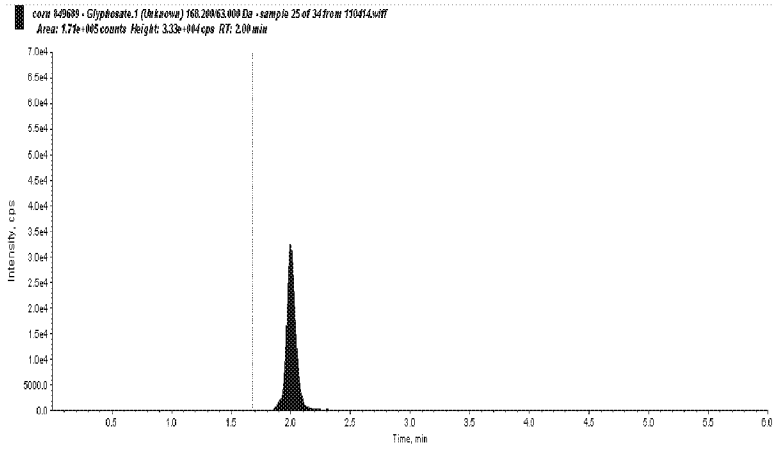
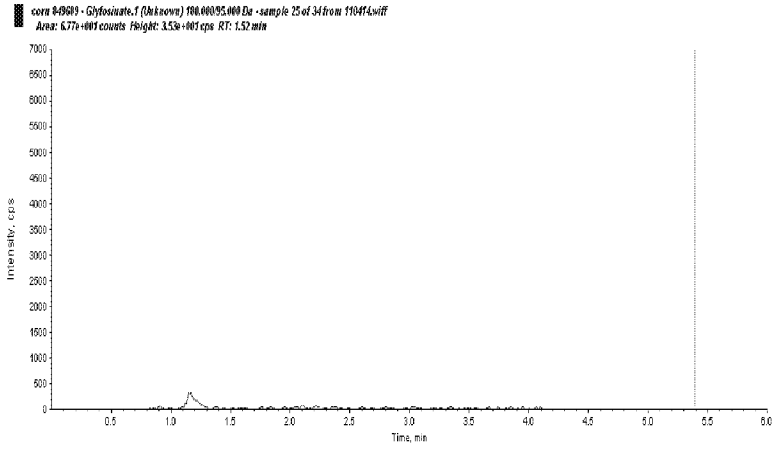


Figure 8 Chromatogram of corn containing 6.5 ppm of glyphosate and 0.065 ppm of AMPA

Glyphosate channel




Glufosinate channel




AMPA channel